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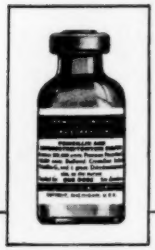

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E D I T O R I A L

AGAIN WE FAIL

PHARMACISTS by and large have again missed a great opportunity to give evidence of their sincerity of purpose and desire to be a full-fledged member of the public health team.

A product was recently offered the public which every thinking pharmacist recognizes as a frank effort to capitalize on the credulity of the American public. In advertising this product the owners would put the old-fashioned "medicine men" of the nineteenth century to shame. While the product is intrinsically harmless it is blatantly sold to the public at a price which is many times its true worth in comparison with many standard products containing essentially the same ingredients.

Some pharmacists have refused to be a party in the sale of this product, recognizing that their obligation to both the public and the medical profession would make such participation impossible. Far too many others, operating on the principle, "Let the buyer beware," feel no compunction—not only in selling it but also in aiding and abetting such sale. The mute evidence of window and counter displays attest to this fact.

There are many pharmacists who attempt to justify their attitude by pointing out that there are a number of established "over the counter" remedies which are in exactly the same category. This is indeed the case but most of these are products of a past era when self-medication was more widely accepted and drugs did not possess the potency and specificity of today's products. They are, furthermore, advertised with some restraint and not as the much sought after "Elixir of Life."

For far too long pharmacists have been attempting to compromise professional integrity with business acumen. At times both objectives are entirely compatible but, when they are not, the former must always take precedence. Those pharmacists who work diligently in promoting good professional relations with medicine, dentistry and nursing, and who give real thought to public health and welfare have every reason to resent the ill-advised actions of many of their fellow pharmacists.

Just imagine the damage done to pharmacy's status when physicians see products of the type in question being advertised and sold by the very persons who clamor for better pharmacist-physician relations and who hail pharmacy as a health profession.

Physicians, today, are utilizing the services of pharmacists more than ever before. Actually, in terms of dollars and cents the pharmacist often receives far more from the patient than does the attending physician. Pharmacists are not in dire economic straits and their prescription volume is better than it has been for years. There is surely no justification for encouraging the public to buy drug items which have little or no benefit and only add to the overall cost of medical care and leave less for real health needs.

If we as pharmacists cannot see our responsibilities and act with restraint then surely we have no right to criticize the dispensing physician or invasions by others into our professional sphere.

We say, "Again We Fail," and yet what an impact we could have made on both the public consciousness and the medical profession had we, to a man, refused to serve as outlets for this twentieth century reincarnation of an "Indian cure-all". We know full well it is wrong, why haven't we the fortitude to stick to our convictions?

L. F. Tice

THE ACTION OF ASCORBIC ACID ON FOLIC ACID *

By Stanley Scheindlin, M.Sc. and Ivor Griffith, Ph.M., D.Sc.

THE chemical interactions between the vitamins of the water-soluble group have long presented a problem to the manufacturer of liquid multi-vitamin products. Since the advent of folic acid it has become evident that this factor of the B-complex also enters into reactions with some of the other vitamins.

In a study of folic acid in liquid prescriptions (1), folic acid was shown to be compatible with many commonly prescribed drugs. It was noted, however, that two liquid polyvitamin mixtures exerted a destructive effect on folic acid after three months' storage at room temperature. This finding tended to confirm the statement of Foss *et al.* (2) that certain factors of the B-complex and also vitamin C have a deleterious effect on folic acid.

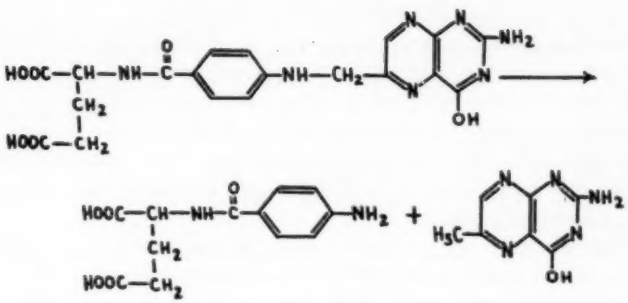
Of interest in this connection are two notes appearing in the recent literature. Gakenheimer and Feller (3) reported that solutions containing vitamin B_{12} and ascorbic acid lost over 50% of their B_{12} activity on standing overnight at 28-30°. Another paper, by Koft and Sevag (4), concerns the loss of folic acid in a neutral culture medium containing (among a variety of other substances) six of the B-complex vitamins. Decomposition of folic acid manifested itself in a liberation of diazotizable amine. These authors did not determine which factors of their culture medium were responsible, but it seems reasonable that the vitamins were among the offenders, if not the sole offenders.

The object of the work to be described in this paper was to determine what chemical reactions occur between folic acid and ascorbic acid in solution.

Method of Approach

It is well known that in acid medium, reducing agents can cleave the folic acid molecule, producing p-aminobenzoyl glutamic acid and 2-amino-4-hydroxy-6-methyl pteridine. The reaction is expressed by the following equation:

* The results of a study undertaken with the aid of a grant from the American Cyanamid Company, Lederle Laboratories Division.



Since ascorbic acid is a reducing agent of some power, it seemed reasonable that this reaction might occur between the two vitamins. If this were the case, the liberated diazotizable amine could be detected by the Bratton-Marshall color reaction.

Experimental

Mixtures of folic acid and ascorbic acid were studied at two pH levels:

1. *Approximate pH 3.5.* This pH is suitable for the stability of most of the B-complex and vitamin C. In most of the experiments at this pH range, 50% by volume of methyl carbitol was used in the solutions, in order to keep the folic acid dissolved. In other solutions, 50% by volume of propylene glycol was employed for the same purpose, although this solvent was unable to keep in solution as high a concentration of folic acid as did methyl carbitol.

2. *Approximate pH 6.5.* This is near the optimum pH for folic acid, and is now considered good for ascorbic acid stability by certain workers (5). At this pH no solvent besides water was used.

In some of the preliminary experiments the two vitamins were reacted by heating in boiling water for various short periods of time. However, most of the studies were carried out by storing the solutions at 42 ± 2° C. for four weeks. Storage at this temperature for three to five weeks is widely considered to be equivalent to a year's storage at room temperature (6) and is used in industry as an "accelerated aging" test in evaluating stability of vitamin products.

All solutions prepared in this entire study were stored in amber bottles, since folic acid is known to be reduced by light (7).

I. Qualitative Experiments

In these preliminary experiments, the Bratton-Marshall reaction was used to test for diazotizable amine following treatment of folic acid by ascorbic and other acids. The procedure used was that given in the chemical assay of folic acid developed by Hutchings *et al.* (8), except that reduction with zinc was omitted. The relative intensity of color produced after diazotization and coupling was judged by visual inspection alone.

The results of the qualitative tests indicated that treatment with ascorbic acid actually produced *less* free diazotizable amine than treatment with other acids at the same pH.

The possibility that ascorbic acid destroyed any diazotizable amine which might be formed was investigated by treating p-aminobenzoic acid with ascorbic acid, storing these solutions at 42°, and testing periodically by the Bratton-Marshall reaction. The content of diazotizable amine definitely diminished.

After the reaction between ascorbic acid and p-aminobenzoic acid had occurred, treatment with zinc and hydrochloric acid failed to liberate any diazotizable amine, as far as could be determined visually.

II. Quantitative Experiments

To confirm and elaborate the qualitative data, reaction mixtures were prepared, aged at $42 \pm 2°$, and assayed quantitatively. The method of Hutchings (8) was used, with the following adaptations:

Diazotization and coupling were carried out in a total volume of 100 cc. rather than 10 cc., to insure greater accuracy. Thus tenfold quantities of reagents were used and volumetric flasks served as reaction vessels.

Diazotization was allowed to proceed for five minutes and destruction of excess nitrous acid by ammonium sulfamate, for three.

Where the original sample was highly colored, blanks were run to account for the color.

Readings were taken in the Klett-Summerson photoelectric colorimeter using a #54 filter.

A curve prepared from reagent grade p-aminobenzoic acid was used as the standard.

A. *Series FT: In acid medium with methyl carbitol*

A stock solution was prepared containing 5 mg. folic acid (by assay) per cc., dissolved with the aid of 1% sodium hydroxide solution.

Gelatin Solution: 0.5% Pharmagel A. and 0.1% benzoic acid. This solution is used in the assay (8) to protect the aromatic amine from partial destruction. The nature of its protection action is unknown and it was used in these solutions quite empirically.

All buffers used in this work were prepared by the Clark and Lubs formulas (9).

INGREDIENTS	SAMPLE No.					
	FT 1	FT 2	FT 3	FT 4	FT 5	FT 6
Folic Acid	25 mg.	25 mg.	25 mg.	25 mg.	25 mg.	
Gelatin Sol.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.
Ascorbic Acid		500 mg.		250 mg.		500 mg.
Citric Acid			500 mg.	250 mg.		
Methyl Carbitol	50 cc.	50 cc.	50 cc.	50 cc.	50 cc.	50 cc.
Buffer, pH 3.4					q.s. 100 cc.	
Distilled water	q.s 100 cc.	q.s. 100 cc.	q.s. 100 cc.	q.s. 100 cc.		q.s. 100 cc.
pH	4.9	3.3	3.2	3.3	4.3	

The solutions were prepared in the afternoon. They stood overnight at room temperature and were assayed in the morning. After assay they were placed in the oven at 42 ± 2° and assayed periodically. For assay, 8 cc. samples were diluted to 100 cc. and 10 cc. portions of this dilution were diazotized and coupled.

After four weeks the samples were submitted for microbiological assay.* The Assay results for this series are given in Table 1.

TABLE I

Sample No.	(Results in mg. per 4 cc.)	Time				Microbiological Assay
		Overnight	4 days	15 days	29 days	
FT 1	Diazotizable Amine Expressed as Folic Acid	0.06	0.23	0.52	0.65	
	Undecomposed Folic Acid	1.03	0.86	0.55	0.40	0.44
FT 2	Diazotizable Amine Expressed as Folic Acid	0.58	0.49	0.34	0.20	
	Undecomposed Folic Acid	0.49	0.52	0.42	0.37	0.34
FT 3	Diazotizable Amine Expressed as Folic Acid	0.06		0.32	0.25	
	Undecomposed Folic Acid	0.99		0.49	0.35	0.33
FT 4	Diazotizable Amine Expressed as Folic Acid	0.76	0.65	0.25	0.09	
	Undecomposed Folic Acid	0.27	0.25	0.17	0.18	0.15
FT 5	Diazotizable Amine Expressed as Folic Acid	0.06		0.40	0.51	
	Undecomposed Folic Acid	1.04		0.65	0.46	0.46

* The authors are indebted to Mr. M. E. Avery, Lederle Laboratories Division, for all the microbiological assays reported herein.

Discussion

It is obvious that the methyl carbitol used, or impurities within it, were able to cleave folic acid even in the absence of ascorbic acid. However, the *course* of the reaction presents interesting variations in each of the solutions studied.

In FT 1 the diazotizable amine content increased progressively, but the sum of the free amine calculated as folic acid and the undecomposed folic acid remained practically constant the whole four weeks. In FT 2, the bulk of folic acid cleavage occurred overnight and then tapered off. An amount of amine equivalent to 0.50 mg. folic acid was lost through further reactions with ascorbic acid or its oxidation products.

In FT 4, the citric acid seemed to accelerate both the cleavage of folic acid and the destruction of the free amine.

A different picture was presented in FT 3, where citric acid alone was present with the folic acid. No free amine was liberated overnight, the sum of the free amine calculated as folic acid and the undecomposed folic acid being 1.05 mg. After four weeks this sum had fallen to 0.60 mg., of which 0.35 mg. was undecomposed folic acid. An amount of aromatic amine equivalent to 0.45 mg. folic acid was thus unaccounted for.

The results in FT 5 were similar to those in FT 1.

B. *Series FP: In acid medium with propylene glycol*

In order to study the decomposition in a less reactive solvent, propylene glycol was next used.

Storage conditions and times of assay for this series were the same as for series FT. For assay 20 cc. samples were diluted to 100 cc. and 10 cc. portions of this dilution were diazotized and coupled.

INGREDIENTS	SAMPLE No.			
	FP 1	FP 2	FP 3	FP 4
Folic Acid	10 mg.	10 mg.	10 mg.	10 mg.
Gelatin Solution	1 cc.	1 cc.	1 cc.	1 cc.
Ascorbic Acid		200 mg.		100 mg.
Citric Acid			200 mg.	100 mg.
Propylene Glycol	50 cc.	50 cc.	50 cc.	50 cc.
Distilled Water	q.s. 100 cc.	q.s. 100 cc.	q.s. 100 cc.	q.s. 100 cc.
pH	7.7	3.1	3.0	3.1

Assay results for the above series are given in Table 2.

TABLE II

Sample No.	(Results in mg. per 10 cc.)	TIME Overnight	2 Weeks	4 Weeks
FP 1	Diazotizable Amine Expressed as Folic Acid	0.03	0.05	0.05
	Undecomposed Folic Acid	1.02	1.01	1.04
FP 2	Diazotizable Amine Expressed as Folic Acid	0.03	0.12	0.06
	Undecomposed Folic Acid	1.06	0.77	0.54
FP 3	Diazotizable Amine Expressed as Folic Acid	0.05	0.03	0.05
	Undecomposed Folic Acid	1.00	1.02	0.99
FP 4	Diazotizable Amine Expressed as Folic Acid	0.05	0.04	0.05
	Undecomposed Folic Acid	1.06	0.96	0.76

Discussion

Propylene glycol exerted no effect on folic acid. The decomposition of folic acid by ascorbic acid took place more slowly than it did in the presence of methyl carbitol. Cleavage of folic acid and degradation of the diazotizable amine occurred almost simultaneously. It was notable that citric acid had no effect when propylene glycol was used, whereas its effect was very marked in the presence of methyl carbitol.

C. *Series FV: Near pH 6.5*

INGREDIENTS	SAMPLE No.					
	FV 1	FV 2	FV 3	FV 4	FV 5	FV 6
Folic Acid	25 mg.	25 mg.		25 mg.	25 mg.	
Gelatin Solution	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.
Ascorbic Acid		500 mg.	500 mg.		500 mg.	500 mg.
Sodium bicarb.		400 mg.	400 mg.		400 mg.	400 mg.
Buffer, pH 6.6				q.s. 100 cc.	q.s. 100 cc.	q.s. 100 cc.
Distilled water	q.s. 100 cc.	q.s. 100 cc.	q.s. 100 cc.			
pH when prepared	7.9	6.7	6.65	6.6	6.55	6.5
pH after 4 weeks	8.1	6.75		6.7	6.4	

Discussion

Inspection of Table 3 reveals that cleavage of folic acid and degradation of the amine occurred almost simultaneously under these conditions. Solutions FV 2 and FV 5 lost 18% and 23%, respectively, of their folic acid in four weeks, while the controls lost only 3% of their folic acid.

D. *Series PAB: Effect of Ascorbic Acid on p-Aminobenzoic Acid.*

Reagent grade PABA was used, in a stock solution containing 1 mg. per cc. in 40% alcohol. Solutions were prepared containing 7.75 mg. PABA (equivalent to 25 mg. folic acid) per 100 cc. They were first assayed after being stored at 42 ± 2° for eleven days, and then two weeks later.

For assay, 8 cc. samples were diluted to 100 cc., and 10 cc. aliquots were diazotized and coupled. Each 10 cc. aliquot theoretically contained 0.062 mg. (62 gammas) PABA.

TABLE III

Sample No.	(Results in mg. per 4 cc.)	TIME 0 Days	14 Days	28 Days	Micro-Biological Assay
FV 1	Diazotizable Amine Expressed as Folic Acid	0.03	0.04	0.05	
	Undecomposed Folic Acid	1.04	1.09	1.01	1.15
FV 2	Diazotizable Amine Expressed as Folic Acid	0.03	0.06	0.08	
	Undecomposed Folic Acid	1.08	1.01	0.89	0.93
FV 4	Diazotizable Amine Expressed as Folic Acid	0.02	0.05	0.05	
	Undecomposed Folic Acid	1.04	1.04	1.01	1.20
FV 5	Diazotizable Amine Expressed as Folic Acid	0.03	0.04	0.07	
	Undecomposed Folic Acid	1.11	1.01	0.86	0.89

INGREDIENTS	SAMPLE No.					
	PAB 1	PAB 2	PAB 3	PAB 4	PAB 5	PAB 6
p-Aminobenzoic acid	7.75	7.75	7.75	7.75	7.75	7.75
Gelatin Solution	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.
Ascorbic acid		500 mg.	500 mg.			500 mg.
Sodium bicarb.						400 mg.
Citric acid				500 mg.		
Methyl carbitol			50 cc.			
Buffer, pH 6.6					equal qu. buffer & water	equal qu. buffer & water
Distilled water	q.s. 100 cc.	q.s. 100 cc.	q.s. 100 cc.	q.s. 100 cc.	q.s. 100 cc.	q.s. 100 cc.

GAMMAS OF PABA

	After 11 days	After Four Weeks
PAB 1	66.2	66.2
PAB 2	10.8	1.5
PAB 3	36.0	14.2
PAB 4	64.3	58.4
PAB 5	65.7	65.7
PAB 6	52.7	32.4

Discussion

These experiments indicate that ascorbic acid and/or its oxidation products react with the $-NH_2$ group of PABA. The reaction proceeded most rapidly in aqueous acid medium, more slowly in 50% methyl carbitol, and still more slowly in water at pH 6.5.

Citric acid did not destroy the diazotizable amine to any great extent. The 64.3 γ recovered after eleven days are not significantly lower than the 66.2 and 65.7 γ of the controls. In the ensuing two weeks, the amine content dropped only 9.2%.

The first time the solutions were assayed, those containing ascorbic acid were treated with zinc to see whether this would release any additional diazotizable amine. The results obtained after treatment with zinc were 10.8 γ for PAB 2, 38.2 γ for PAB 3, and 52.7 γ for PAB 6. Thus, after the aromatic amine has reacted with ascorbic acid, it is not regenerated by zinc and hydrochloric acid.

Conclusions

1. The decomposition of folic acid by ascorbic acid occurs in two stages;

(A) Cleavage of the folic acid molecule, yielding a pteridine as well as p-amino benzoyl glutamic acid.

(B) Destruction of the diazotizable amino group of the latter compound by ascorbic acid or its oxidation products.

2. The reactions proceeded most rapidly in the presence of 50% methyl carbitol as a solvent, since methyl carbitol itself or substances within it facilitated the cleavage of folic acid.

3. With methyl carbitol present, citric acid accelerated the reactions between folic acid and ascorbic acid. This effect of citric acid was not present when propylene glycol replaced the methyl carbitol in the solvent.

4. When the solvent was 50% propylene glycol, folic acid decomposition occurred more slowly, and the two stages of the reaction were nearly simultaneous, not clearly separated as they had been in methyl carbitol.

5. At about pH 6.5, in aqueous medium, the interaction between the two vitamins proceeded slowly, only about 20% of the folic acid having been cleaved in four weeks.

All the reactions reported herein occurred under the accelerated aging conditions described in this paper.

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IODINE AS A SANITIZING AGENT FOR FOOD AND EATING UTENSILS *

By Louis Gershenfeld and Bernard Witlin**

THE sanitization of food and eating utensils is of great importance in public health work today. The existence of unsatisfactory conditions has been aggravated by the laxity of those directly concerned with this problem. McCulloch (14) indicated that "most restaurants and, particularly, soft drink stands and saloons have no provision for adequate sanitization of utensils used in the serving and eating of foods. Forks, spoons, and glasses which have been brought in intimate contact with the mouth of one customer are frequently used by another after only the most superficial rinsing to remove particles of nutriment. Many of the glasses from which drinks are sold do not have the advantage of even the physical removal of adhering mucus to the rims. The condition is unfortunately tolerated because it is so commonplace, however, it is now apparent that this great carelessness is responsible for the transmission of many infectious diseases. The problem is now receiving more serious consideration."

More recently Graber and Miller reported that aboard ships proper treatment of eating and drinking utensils was not practiced (19).

Among the halogens, sodium hypochlorite solutions and solutions of free chlorine have been used as sanitizing agents for the washing of surfaces of food equipment (14)(16)(17). Solutions containing free iodine are known to be active and reliable bactericides (14). The concentration of free iodine necessary to disinfect does not vary greatly with different organisms (5). Free iodine also has been shown to possess viricidal activity (2)(3)(4)(9)(12)(18).

Purpose

The purpose of this study was to determine the efficiency of solutions of free iodine as sanitizing agents for eating and drinking utensils.

---

* This investigation was conducted at the Philadelphia College of Pharmacy and Science under a grant from the Chilean Iodine Educational Bureau's Fellowship, Mellon Institute. Presented before the American Public Health Association Laboratory Section Meeting, St. Louis, Mo. November 3, 1950.

** Department of Bacteriology, Philadelphia College of Pharmacy and Science.

Technique

The testing procedures used in these experiments were the method devised by Johns (10) (11), which endeavors to approximate the conditions under which a sanitizing solution performs its work in actual practice.

Experimental—Part I

A 20 to 24 hour old growth of *Escherichia coli* on F. D. A. agar medium (16) grown at 37° C. was suspended in sterile isotonic sodium chloride (0.9%) solution. After filtration through sterile Whatman #1 filter paper, the suspension was standardized against a barium sulfate turbidity standard to give a concentration of 300 million bacteria per ml. (15). One ml. of this suspension was then introduced into 60 ml. of a 1:10 dilution of sterile skim milk. The diluted skim milk had been previously autoclaved at 121° C. for 15 minutes.

The diluted skim milk-bacteria suspension as prepared above was placed in a small sterile bowl (Fig. 1) to a depth of 1.5 inches. With sterile forceps, a previously sterilized glass slide (individually wrapped in paper and sterilized in the hot-air oven at 170° C. for 2 hours) was dipped into the seeded skim milk suspension to a depth of approximately 1.5 inches. The lower half of the slide was then drained against the rim of the bowl for approximately 10 seconds to remove the excess liquid. The slide was finally placed on end on a pad of sterile filter paper in a specifically constructed draining can (Fig. 2).

One hundred ml. portions of the solutions of disinfectant to be tested were placed in sterile 150 ml. beakers. The treated slides were drained of excess liquid on the filter paper layers in the draining can, dipped into the solutions of bactericide under test, and gently agitated for the required period of time. They were then momentarily dipped into sterile tap water contained in a beaker to rinse and thereby minimize bacteriostatic action of the adhering bactericides, shaken once to remove excess liquid and placed in a Petri dish. The plate was immediately poured with F. D. A. agar (16) containing 1% sodium thiosulfate (as a halogen inactivating agent), mixed, allowed to solidify, and incubated at 37° C. for 24 hours.

Control plates were prepared at regular intervals during each day's testing, using the same technique except that there was no treatment with solutions of bactericides.

FIGURE 1
Bowl Used for Diluted Skim Milk-Bacteria Suspension

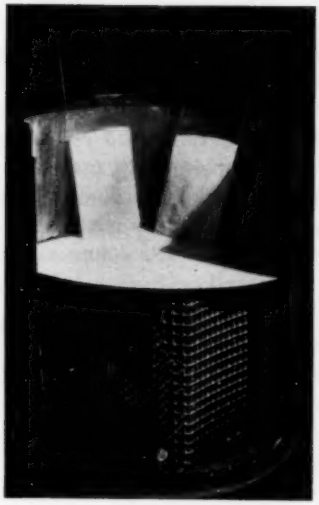

FIGURE 2
Draining Can, Front Panel Removed to Show Draining of Skim Milk-Bacteria Coated Slides

Exposure times were selected as 1, 10 and 20 seconds. This critical time period was used, because during rush hours dishwashers usually give the utensils and glassware an instantaneous dip to perhaps a 10 second wash.

The stock solution of antibacterial agent was Iodine Solution N. F. IX (containing 2% free iodine).

The efficiencies of all halogens have been observed to be markedly reduced by the presence of organic material (10). The skim milk used in the testing technique contained protein and other organic matter which most likely would be encountered in the sanitization of food utensils.

The experiments in Part I were conducted employing various dilutions of free iodine. The dilutions ranged from 200 ppm. down to 0.5 ppm. The 200 ppm. free iodine solutions were prepared by placing 1 ml. of (2%) Iodine Solution in 99 ml. of distilled water. Weaker dilutions were made using this 200 ppm. free iodine solution.

Findings—Part I

As noted in Table I, when organisms (*E. coli*) suspended in diluted skim milk and coated on glass slides were exposed to solutions of free iodine with concentrations varying from 25 ppm. to 200 ppm., growth was not observed at any of the exposure time periods investigated (1, 10 and 20 seconds). However, a suspension-coated slide immersed in 10 ppm. free iodine showed one colony in the 10 second dip and 18 colonies in the one second dip as compared with the controls, which yielded 286 colonies following the one second immersion and 195 colonies following the 10 second immersion.

TABLE I

OBSERVATIONS ON AGAR PLATES AFTER IMMERSION IN SOLUTIONS OF FREE IODINE

Slides coated with suspension of organism (*E. coli*) in skim milk	Concentration of free iodine in solutions used					
Time of exposure of slide in solution (in seconds)	200 ppm.	100 ppm.	50 ppm.	25 ppm.	10 ppm.	Control
	Average No. of colonies					
1	0	0	0	0	18	286
10	0	0	0	0	1	195
20	0	0	0	0	0	158

Room Temp: 23°C. 0: No growth

As noted in Table II, when organisms suspended in diluted skim milk on sterile slides were immersed in solutions of 1, 2, 3 and 4 ppm. free iodine, colonies (always in excess of 100) developed after treatment in all dilutions and at all time intervals investigated (1, 10 and 20 seconds). Control plates averaged 480 colonies following a one second dip of the suspension-coated slide, 280 colonies after the 10 second dip, and 150 colonies after the 20 second dip.

TABLE II

OBSERVATIONS ON AGAR PLATES AFTER IMMERSION IN SOLUTIONS OF FREE IODINE

Slides coated with suspension of *E. coli* in skim milk	Concentrations of free iodine in solutions used				
Time of exposure of slide in iodine solution (in seconds)	1 ppm.	2 ppm.	3 ppm.	4 ppm.	Control
	Average No. of colonies				
1	146	260	145	194	480
10	310	290	260	243	280
20	190	112	122	132	150

Experimental—Part II

Experiments were conducted on free iodine solutions to determine their effectiveness as sanitizing agents and to note their stability when they were used for periods of ½, 4, 8, 16, 24 and 48 hours after preparation.

Two hundred ml. portions of solutions of free iodine (50, 100, 200 and 400 ppm.) were prepared by diluting solutions of 2% iodine. The 200 ml. portions of the test solutions were placed in 250 ml. beakers previously sterilized in the hot air oven for at least 1 hour at 170° C.

Using the Johns' (10) testing method described under Part I, suspension-coated slides of *E. coli* and skim milk were then immersed in the above solutions for periods of 1, 10 and 20 seconds. The slides were placed in Petri dishes, covered with F. D. A. agar (16), allowed to solidify, and incubated at 37° C. for 24 hours. The colonies on the plate were then counted. As controls, the same technique was used except that there was no treatment with the solutions under test.

Test solutions of 50, 100, 200 and 400 ppm. free iodine were used. These same solutions were also tested, ½, 4, 8, 16, and 24 hours after preparation. After each test, 10 ml. samples of each solution used were examined for free iodine remaining in the solutions.

Findings—Part II

As noted in Table III, slides coated with *E. coli* suspended in diluted skim milk and immersed for 1, 10, and 20 seconds in 100 ppm. free iodine solutions, which were ½ and 4 hour old, did not reveal the presence of bacteria. Control plates for the one-half hour test always yielded 250 colonies or more in the 1, 10 and 20 second immersions. Control plates for the 4 hour test solution always showed 200 colonies or more in the 1, 10 and 20 second immersions.

Suspension-coated slides immersed for one second in 16 hour-old solutions which originally contained 100 ppm. free iodine revealed the presence of 14 colonies as compared with the control which yielded 102 colonies. A suspension-coated slide immersed for 10 seconds in the 16 hour-old solution revealed the presence of one colony as compared to the control which yielded 220 colonies. A suspension-coated slide immersed for 20 seconds revealed no colonies, as compared with the control which yielded 290 colonies.

TABLE III

SANITIZING EFFECT OF FREE IODINE (100 ppm.) ON *E. coli*

Age of solution used in tests at room temp. (23°C.)	Average number of colonies on agar plates after immersion in iodine solution. Time (in seconds) of exposure of slide in solution of bactericide						Conc. of iodine in sol'n after contact with suspension coated slides ppm.
	1	Control	10	Control	20	Control	
Immed. after 1st test (approx. ½ hr.)	0	365	0	330	0	260	88
4 hr.	0	200	0	310	0	212	63
16 hr.	14	102	1	220	0	290	11
24 hr.	4	250	5	285	1	125	7

TABLE IV

SANITIZING EFFECT OF FREE IODINE (50 ppm.) UPON *E. coli*

Age of solution used in tests at room temp. (24°C.)	Average number of colonies on agar plates after immersion in iodine solution. Time of exposure of slide in solution of bactericide (in seconds)						Conc. of iodine in sol'n after contact with suspension coated slides ppm.
	1	Control	10	Control	20	Control	
Immed. after 1st test (½ hr.)	0	267	0	243	0	200	39
4 hr.	0	355	0	287	0	226	28
8 hr.	0	350	1	290	2	231	12
16 hr.	100	362	10	420	25	207	None detected
24 hr.	120	265	75	293	20	220	None detected
38 hr.	335	375	235	230	287	200	None detected

The 1 second immersion of the suspension-coated slide in the 24 hour-old solution which originally contained 100 ppm. free iodine showed 4 colonies as compared to the control which yielded 250 colonies. A similar test using the 10 second immersion in the 24 hour-old solution revealed the presence of 5 colonies as compared to the control which yielded 285 colonies. The 20 second dip interval produced one colony, as compared to the control which yielded 125 colonies.

An analysis of the test solution, containing originally 200 ppm. free iodine, showed that 88 ppm. free iodine remained in the solution after one-half hour. At the end of 4 hours, the solution contained 63 ppm. of free iodine. Following 16 hours of exposure, 11 ppm. free iodine remained and after 24 hours the free iodine present in the solution was 7 ppm.

As noted in Table IV, a suspension-coated slide immersed for one second in a one-half hour-old 50 ppm. (original) free iodine solution yielded no colonies, as compared to the control which revealed the presence of 267 colonies on the agar plate. Tests conducted for 10 and 20 second immersion periods in the above solution revealed no colonies as compared to the controls which showed 243 and 170 colonies.

When a 4 hour-old 50 ppm. (original) free iodine solution was examined, the one second dip of the *E. coli* coated-slide produced no colonies, as compared to the control which had 355 colonies. Similar tests on a 4 hour solution, using a 10 and 20 second dip of the slides, revealed no colonies, while the controls yielded over 200 colonies.

Tests conducted on an 8 hour-old 50 ppm. (original) free iodine solution showed one colony when the suspension-coated slide was immersed for 10 seconds and two colonies when immersed for 20 seconds. Control plates always showed more than 200 colonies.

The 16 and 24 hour-old original 50 ppm. free iodine solutions were not as effective as the ½ to 8 hour-old solutions. The one second immersion of the suspension-coated slide in the former solutions yielded over 100 colonies in both instances. Control plates revealed more than 200 colonies. The 10 second immersion of the suspension-coated slide in the 16 hour-old solution yielded 10 colonies, as compared to 420 in the control. In the case of the 24 hour-old solution, the 10 second immersed slide yielded 75 colonies. When the 20 second dip of the slide was used in the 16 and 24 hour-old solutions,

both yielded 20 colonies as compared to over 200 counted on the control plates.

An investigation of the free iodine solution after the ½ hour test revealed that 39 ppm. free iodine remained. At the conclusion of the 4 hour test, 28 ppm. free iodine was present and 12 ppm. remained in solution 8 hours after preparation. Solutions which were 16, 24 and 48 hours old did not reveal the presence of free iodine.

As noted in Table V, suspension-coated slides immersed for one second in a ½ hour-old 200 ppm. free iodine solution yielded no colonies, as compared to the controls which yielded an average of 251 colonies. Similar tests conducted on 8 hour-old and 24 hour-old 200 ppm. free iodine solutions also revealed no growth while controls showed 137 and 204 colonies.

E. coli coated slides tested at 10 and 20 second intervals in solutions which originally contained 200 ppm. free iodine yielded no colonies, after exposure at ½, 8 and 24 hours, as compared to control plates which revealed between 135 and 250 colonies. It was found that at the end of ½ hour, 171 ppm. of free iodine remained in the test solutions. At the end of 8 hours, 67 ppm. of free iodine remained; and at the end of 24 hours, 18 ppm. of free iodine were found in the test solutions.

As noted in Table VI, suspension-coated slides immersed for one second in 400 ppm. free iodine solutions which were ½, 8 and 24 hours old revealed no colonies, while control plates showed between 158 and 266 colonies.

Colonies were not found in the 10 second or 20 second immersions of the suspension coated slides in 400 ppm. free iodine solutions which were ½, 8 and 24 hours old. Control plates showed from 177 to 300 colonies.

An analysis of the test solutions at the end of ½ hour revealed the presence of 379 ppm. of free ioidne. When the 8 hour samples were examined, 189 ppm. of free iodine were found; and at the end of the 24 hour tests 30 ppm. of free iodine remained in the test solutions.

Experimental—Part III

In most homes and in many public eating and drinking establishments, dishes are washed manually. Accordingly studies were made to note the sanitizing efficiency of iodine for eating utensils following soil removal and manual washing. Cafeteria dishes, cups, glassware,

TABLE V

SANITIZING EFFECT OF FREE IODINE (200 ppm.) ON *E. Coli*

Age of solution used in tests at room temp. (23°C)	Average number of colonies on agar plates after immersion in iodine solution. Time of exposure of slide in solution of bactericide (in seconds)						Conc. of iodine in sol'n after contact with suspension coated slides ppm.
	1	Control	10	Control	20	Control	
Immed. after 1st test (½ hr.)	0	251	0	181	0	233	171
8 hr.	0	137	0	180	0	230	67
24 hr.	0	204	0	135	0	176	18

TABLE VI

SANITIZING EFFECT ON IODINE (400 ppm.) ON *E. Coli*

Age of solution used in tests at room temp. (24°C.)	Average number of colonies on agar plates after immersion in iodine solution. Time of exposure of slide in solution of bactericide (in seconds)						Conc. of iodine in sol'n after contact with suspension coated slides ppm.
	1	Control	10	Control	20	Control	
Immed. after 1st test (½ hr.)	0	243	0	300	0	216	379
8 hr.	0	266	0	247	0	197	189
24 hr.	0	158	0	221	0	177	30

knives, forks, and spoons after scraping and a preliminary 10 second spray rinse in water (between 130° F. and 170° F.), were sanitized by dipping in iodine solutions for 1, 10, 20, 30 and 60 seconds intervals (100 ppm. and 200 ppm.).

Findings—Part III

In all instances the results were identical and comparable (killed all organisms following a 1 second immersion) to those obtained with the skim milk-bacteria coated glass slides.

Summary and Conclusions

1. (a) A suspension of *E. coli* (300 million per ml.) was prepared in sterile isotonic sodium chloride solution. One ml. of this suspension was introduced into 60 ml. of a sterile 1:10 dilution of skim milk. A sterile glass slide was dipped into the skim milk-bacteria dilution to a depth of 1.5 inches, drained in a sterile container to remove excess liquid and immersed for varying time intervals (1, 10 and 20 seconds) in solutions of free iodine of known concentrations. The slides were placed in sterile Petri dishes, covered and mixed with F.D.A. agar. The agar plates were incubated at 37° C. for 24 hours and the number of colonies counted. Control plates were prepared using the same technique except that there was no treatment with the solutions under test.

1. (b) Cafeteria dishes, cups, glassware, knives, forks and spoons which had been scraped and given a preliminary 10 second rinse in water (between 130° F. and 170° F.) were sanitized by a one second immersion in iodine solutions (100 ppm. and 200 ppm.).

The dilutions of free iodine tested ranged from 200 ppm. to 0.5 ppm. Solutions containing free iodine were prepared from Iodine Solution (2%) N. F. IX. The 200 ppm. free iodine dilution was prepared by placing 1 ml. of the 2% iodine solution in 99 ml. of distilled water.

2. Suspension-coated slides immersed in 4 ppm. free iodine solutions for 10 seconds yielded an average of 243 colonies as compared to a 280 colony average for the controls. The 20 second immersion of the slides produced an average of 132 colonies as compared to the 150 colony average observed on the controls.

3. Tests carried out on 50 ppm. free iodine solutions at ½, 4 and 8 hours after their preparation, by dipping suspension-coated slides in these solutions for 1, 10 and 20 seconds did not produce more than 5 colonies on any test plate as compared to controls which yielded between 231 and 355 colonies. Twelve ppm. of free iodine remained in solution at the end of the 8 hours after preparing the solutions. Investigations conducted on 16, 24 and 48 hour old 50 ppm. free iodine solutions yielded 20 to 335 colonies. Control plates yielded between 207 and 420 colonies. Free iodine was not detected in the test solutions after 18 hours.

4. *E. coli*-coated slides immersed for 20 seconds in 16 hour-old 100 ppm. free iodine solutions yielded no colonies. Controls yielded over 200 colonies per plate. Similar tests (20 seconds) on solutions which were 24 hours old showed an average of one colony, as compared with controls which averaged 125 colonies. An analysis of the test solutions at the completion of the 24 hour test established that 7 ppm. of the original 100 ppm. free iodine remained in the solution.

5. *E. coli*-coated slides immersed for either 1, 10 or 20 seconds in both 200 ppm. and 400 ppm. free iodine solutions yielded no colonies as compared to controls which yielded between 135 and 251 colonies in all cases. The ages of the solutions tested were ½ hour and 24 hours. An analysis of the 200 ppm. free iodine solutions at the conclusion of the 24 hour test revealed that they retained 18 ppm. free iodine. Similar tests on the 400 ppm. free iodine solutions at the end of the 24 hours showed that they contained 30 ppm. free iodine.

6. It was observed that solutions originally containing 200 ppm. of free iodine still contained 67 ppm. of free iodine after 8 hour periods. These solutions inhibited growth on the slides in 20 second immersions; identical results were produced in the 1 and 10 second immersions.

7. Solutions containing 100 ppm. of free iodine killed all organisms on the slides and on eating utensils in a 20 second immersion. They therefore fulfill the requirements set forth by the United States Public Health Service "Code Regulating Eating and Drinking Establishments" and may be recommended for use in food utensil sanitization.

8. The use of 200 ppm. solutions of free iodine for the sanitization of food utensils is suggested. This concentration gives greater safety throughout the 8 hour working day. A teaspoonful of Iodine Solution (2%) (N. F. IX) or Iodine Tincture (2%) (U.S.P. XIV) to a pint of water will give a solution of approximately this strength. Solutions of this concentration were found to have no adverse effect upon any kind of utensils after repeated treatments.

It is possible to quickly prepare free iodine solutions containing 200 ppm. for use everywhere including its use in the home when sanitization is to be practiced for eating utensils used by the sick.

9. Solutions of free iodine of sufficient strength to be used safely in food utensil sanitization were observed to range in color from yellow to deep amber, while solutions too dilute to inhibit the growth of the test organism were very pale yellow. This change in color can be used to advantage by the layman in judging whether solutions of free iodine still possess their sanitizing strength.

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COMPARISON OF METHODS FOR OBTAINING BLOOD FROM MICE *

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THERE are many satisfactory methods for obtaining blood from rats (2, 3) and most of the other small laboratory animals. However, as to our knowledge there are no uniform, satisfactory methods generally accepted for mice.

In a series of over 200 mice, different methods of taking blood were compared. Swiss male mice were used of our own colony of 25 ±2 Gm. body weight. They were kept in air-conditioned quarters at 21° C., 48% relative humidity and were maintained on Rockland rat pellets and water *ad lib.* Previous trials showed that females generally yield less blood than males of similar weight. On the other hand, with males it is easier to avoid contamination with fecal matter in the collecting tubes when blood is taken by the tail-snipping method. Blood was collected in small graduated tubes. If plasma or blood cells were desired, 0.2 mg. heparin or 0.2 ml. 3.8% sodium citrate was previously placed into the tubes and gently mixed with the blood after collection. Hematocrit readings were performed with van Allen microhematocrit tubes according to the method described by the author (1).

Methods for obtaining mixed venous and arterial blood. The most frequently used method is decapitation under ether anesthesia. An assistant holds the skin over the head of the deeply anesthetized animal with a hemostat, and the operator quickly decapitates with a pair of sharp scissors. Free flowing blood is then collected in a tube. An average of 0.6 ml. of blood per mouse can be obtained in this way. An advantage of this method is its rapidity but a disadvantage is the possible admixture of other body fluids. Furthermore, the mouse is destroyed.

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Another often-used method is to cut the tail of the animal. According to our experience the largest amount of blood can be obtained, if the tail is cut close to the body. Cutting the tail near the end and progressively toward the body until blood is no longer obtained resulted in a smaller total quantity. Previous immersion in a 45° C. bath for 1 minute, wiping with xylol and subsequently with alcohol or a combination of these two procedures did not increase the flow of blood if the tail was cut close to the body. Ether anesthesia definitely increased blood flow, this increase being greater with deeper anesthesia. To further increase flow by suction, a simple apparatus was constructed. A graduated tube to collect blood is placed inside a 12 cm. long, 1.5 cm. diameter pyrex tube with a side arm. Through a rubber stopper a glass tube of gradually decreasing diameter is inserted (upper diameter: 5 mm., lower diameter: 2.5 mm.). (Fig. 1.) The tail stump is then inserted in this tube, and gentle suction applied to the side arm. Suction causes the tail to seat tightly in the tube resulting in an outflow of additional blood. An apparatus employing suction to obtain blood has been described by Sjöwall (4) for rabbits; Plum (3) for rats, and others have used similar procedures on the ears of guinea pigs.

To maintain the mice under deep anesthesia during the procedure and to enable an individual to perform the entire operation, a small aluminum foil funnel is mounted on a stand about 40 cm. over the table top, into this funnel is placed cotton moistened with ether. The operator holding the collecting tube in his left hand and the mouse in his right hand can move the mouse under or away from the funnel, thus maintaining uniform, deep anesthesia (Fig. 1). A suitable over-all procedure is as follows: The mouse is deeply anesthetized with ether, his tail cut about 1.25 cm. from the body and blood collected in the graduated tube. If bleeding is insufficient, the stump is wiped with cotton moistened with 3.5% sodium citrate or 10 mg.% heparin in 0.9% sodium chloride solution, to remove clots. Then if necessary the stump is placed in the suction tube and further amounts are withdrawn. In this manner an average of 0.75 ml. of blood per mouse can be obtained. Mice not under anesthesia yield an average of 0.6 ml. of blood. After bleeding, animals may receive 2 ml. of 0.9% sodium chloride intraperitoneally. Death rarely occurs. Blood values return to normal within two weeks at which time the procedure may be repeated, cutting the remaining stump from the body at about 0.8 cm.

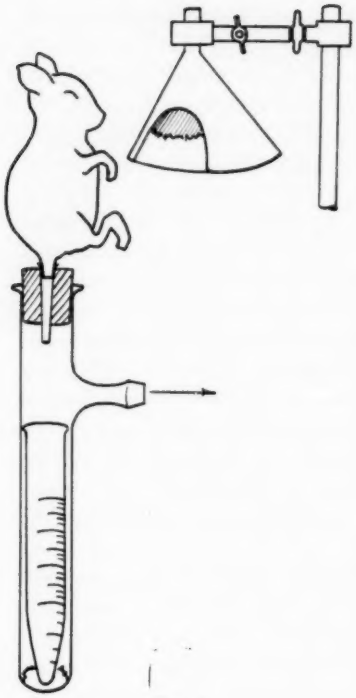

FIG. 1

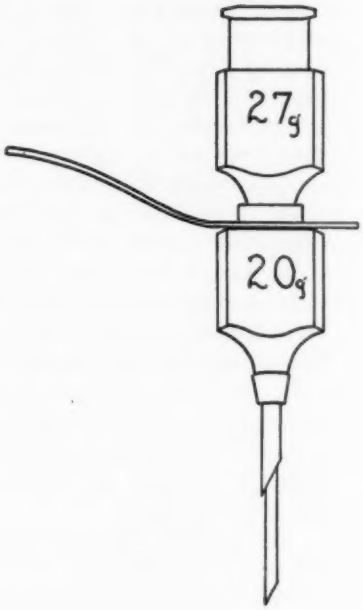

FIG. 2

To remove maximal amounts of blood from the animal the following method may be used: The deeply anesthetized animal is fastened to an operation board mounted about 20 cm. high over a table top, the femoral artery and vein are dissected. A cannula (made from a 27 gauge hypodermic needle) is bound into the femoral vein (if this operation is not successful on either side, the jugular vein is used), a 20 cc. syringe containing 10 mg.% heparin in Tyrode solution or in 0.9% NaCl warmed to 37° C. and saturated with oxygen is attached to the cannula, the femoral artery is then cut and the outflowing blood is collected in a graduated tube placed beside the operation board. The board is slightly tilted toward the tube and adjusted so that all free flowing blood can be collected. After collecting about 0.2 ml. of blood, the graduated tube is replaced with a second graduated tube. Now slow infusion of the solution in the

syringe is started and continued until the outflowing fluid becomes clear or the heart stops. Hematocrit values are taken of the blood in both collecting tubes. From this data the dilution factor of the blood in tube II can be calculated. Similar approximate information can be deduced from the total volume obtained in tube II and the amount of fluid injected.

Methods for obtaining pure venous or pure arterial blood. The anesthetized animal is mounted on the operation table. The jugular veins are exposed on both sides. The vein on one side is closed with a clip and that on the other side is cut, from which blood is collected as above. An average of 0.75 ml. can be obtained. After this procedure the cut jugular vein is ligated, the clamp removed from the other vein and the wound closed with a suture, then 2 cc. of 0.9% sodium chloride are injected intraperitoneally. This operation can be repeated on the survivors two weeks later, utilizing the remaining jugular vein. The analogue of this procedure can be accomplished with the carotid arteries, thus obtaining arterial blood.

Method for obtaining sterile blood. To obtain sterile blood, heart puncture is the most satisfactory procedure. The hair of the mouse is clipped with electric clippers over the cardiac region, the area subsequently shaven with a razor, and the skin painted with diluted iodine tincture. At the point of the maximal heart beat (about a quarter to a half finger over the xyphoid process on the right side of the sternum), a sterile 20 gauge needle (cut down to 6 mm. length and sharpened to have a short bevel and mounted on a metal handle, Fig. 2) is inserted through the skin. Through this needle a sterile 27 gauge 1 in. needle is inserted into the heart and blood withdrawn into a sterile tuberculin syringe. A volume of 0.2 ml. can be obtained in this way without seriously affecting the animal.

Methods for obtaining small amounts of blood for blood counts. For this purpose the tail is immersed in 45° C. water for 1 minute, wiped with xylol, alcohol and dried, then cut near the tip with or without ether anesthesia. The first drop of blood is wiped off with nonabsorbent cotton. The operator takes blood from the subsequent drop into the blood counting pipette. If coagulation occurs, an assistant wipes the wound with nonabsorbent cotton moistened with 10 mg.% heparin in 0.9% sodium chloride solution. If bleeding is more rapid than required or the operator changes pipettes, blood is

collected in a small paraffined watch glass containing 0.2 mg. of heparin powder and gently stirred with a glass rod. Bleeding may be controlled by pressing absorbent cotton on the wound. Three samples taken for blood counts within two hours did not show significant changes in the results obtained. In the few cases where this procedure is not successful, blood can be obtained under ether anesthesia by cutting the tail higher or by heart puncture. Anesthesia of medium depth did not alter blood values.

Obtaining blood from embryos or new born mice. Amputation of a limb is the most satisfactory method, according to our experience. Blood is received into a small tube which may contain heparin, the amount collected is governed in a large measure by the age of the subject.

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SELECTED ABSTRACTS

The Clinical Use of Radioactive Isotopes. Lawrence, J. H. *The Bull. N. Y. Acad. Med. 26:*639 (1950). The advent of radioactive isotopes has opened two fields of usefulness in the medical realm. The field of tracer technique developed most for the radiations emitted by various radioactive isotopes such as phosphorus, iron, sodium, hydrogen, carbon and others, can be traced within the intact human body or experimental animal as well as in the laboratory test tube. The nature of such radioactive elements is altered only as to its radioactivity. To a lesser degree of usefulness these isotopes have provided a means of supplying radiation for use in therapy in place of radium and the X-ray machine.

The isotope P^{32} has been found useful in labeling red blood cells in tracer studies to determine the blood volume. This determination has been shown to be a reliable and simple method of diagnostic usefulness in a variety of clinical conditions where red blood cell counts and hematocrit determinations are often misleading. P^{32} labeled red cells have proven to be valuable also as a means of evaluating the degree of dilution of a sternal marrow puncture with peripheral blood. Correction can thus be made for this dilution in the interpretation of the findings. The use of water labeled with H^3 has shown promise as a method for the determination of total body water by the dilution of the activity injected in studies of the body water in such conditions as heart failure, kidney disease, and similar diseases. The rates of mixing of radioactive water, radioactive carbon monoxide, radioactive sodium chloride, and P^{32} labeled red blood cells in normal individuals as compared with edematous and nonedematous patients with heart disease and nephritis give a measure of the dynamics of circulation in such persons.

Further applications of the tracer technique have been applied in the rate at which Fe^{59} is cleared from the plasma and incorporated into red blood cells under various diseased conditions. In leukemia there is apparently not only a rapid white cell production but also a normal or rapid red cell production as evidenced by the normal or rapid rate of iron incorporation into red cells. Tracer studies also

showed that instead of a maturation arrest of red cell formation in pernicious anemia there is apparently an abnormality about the length of life of red cells, for iron was being incorporated into red cells at a normal rate. Other studies have made use of C^{14} to detect the presence of carcinoma, radioactive sodium and radioactive inert gases to study the circulation in the extremeties, and radioactive sodium chloride solution to study the dynamics of blood flow through the chambers of the heart.

Because of the danger involved in therapy with radioactive materials the author stated that no artificial radioactive material should be used in therapeutic doses in any patients other than those with a relatively short life-expectancy. Therefore, leukemia was the first disease treated by the author. He found that P^{32} did become concentrated to a relatively high degree in rapidly growing leukemic tissue and in bone marrow. The use of this isotope had resulted in an average life-expectancy of 17 years among 150 patients treated by the author. This compares favorably with a life-expectancy of 14 years in a disease such as diabetes. Unusually favorable results were obtained in some cases of giant follicle lymphoblastoma and multiple myeloma using P^{32} as the therapeutic agent but little success was obtained in the treatment of Hodgkin's disease. Treatment of superficial skin cancers with this isotope applied locally to a pad cut exactly to the shape of the lesion has met with considerable success. Radioactive iodine was found to localize in certain types of metastatic thyroid cancer. In such cases it has been possible to obtain palliation of the disease and extension of a comfortable life.

In conclusion the author pointed out that the major application of radioisotopes to medicine must be to the understanding of physiological and pathological processes with an alertness to possible therapeutic applications to diseases not successfully treated at the present time.

Hypercoagulability of the Blood During Therapy With ACTH and Cortisone. Cosgriff, A. W., Diefenbach, A. F., and Vogt, W., Jr. *Am. J. Med. 9:752* (1950). The occurrence of a number of thromboembolic complications in patients under treatment with ACTH or cortisone led the authors to study the possible increase in clotting tendency of patients receiving these substances.

Venous clotting times studied in 10 patients revealed that in 8 of these there was considerable shortening of the clotting time while the patients were receiving ACTH or cortisone. Heparin-retarded venous clotting time studies revealed a similar state in 4 of 6 patients. This hypercoagulatility was maintained in 3 patients for from 7 to 15 days after discontinuation of therapy. The degree of hypercoagulability may have a direct relationship to dosage for it was noted that one patient receiving 25 mg. of ACTH every 6 hours showed a moderate hypercoagulability which decreased when the dosage was decreased to 12.5 mg. every 6 hours. With equal doses of ACTH and cortisone, ACTH appeared to cause a greater hypercoagulability.

The hypercoagulability was controlled with heparin and dicumarol. There was no evidence of a significant change from normal in the response to heparin among patients under ACTH or cortisone therapy. With dicumarol there seemed to be an increase in anticoagulant effect among these patients.

The mechanism of the altered state of blood coagulation during ACTH and cortisone therapy was not clear from these studies. But, the authors stated that the presence of factors which in themselves may predispose to intravascular thrombosis may combine with the hypercoagulability secondary to ACTH or cortisone administration to precipitate intravascular coagulation. In such conditions it would appear to be wise to employ prophylactic anticoagulant therapy.

Intra-Arterial Histamine in the Treatment of Claudication and Rest Pain. Mackey, W. A. *Brit. Med. J.* No. 4688:1086 (1950). When the main vessel of a limb is obliterated by thrombosis the blood supply to points distal to the thrombus depends upon the adequacy of the collateral network of vessels. The general aim of treatment in such cases is to aid and augment the development of the collateral vessels, since the blockage in the main vessel is often relatively permanent. Vasodilation is the principle employed to accomplish the desired end. The methods employed to accomplish this are often either insufficient or produce too general an effect. The author described a method for obtaining a comparatively localized effect on 14 patients under his care. A dilute solution of histamine acid phosphate was introduced into the femoral artery at the groin under a pressure between the diastolic and systolic pressures of the patient.

The solution was made by dissolving 2 milligrams of histamine acid phosphate in 500 cc. of normal saline solution. This was then administered over a period of 30 to 45 minutes. Ten such infusions spaced at intervals of 1 week constituted the usual treatment schedule.

A successful treatment was evidenced by improved color in the limb and a rise in skin temperature of the toes. Nine of the patients showed subjective and clinical improvement as evidenced by increased capacity for walking and a diminution of "rest pain". The other 5 patients showed no evidence of improvement but in no case was there evidence that the treatment had done any harm. The dose of histamine, although capable of producing marked systemic effects if introduced into the general circulation, is apparently used almost entirely by the tissues of the limb for the general effects were found to be slight.

Important Factors in Aerial Disinfection With Triethylene Glycol Vapor. Lester, W., Jr., Kaye, S., Robertson, O. H., and Dunklin, E. W. *Am. J. Pub. Health 40:*813 (1950). Under controlled laboratory conditions triethylene glycol vapor has been found to exert a rapid lethal action against many air-borne infectious agents. The bacteria found to be susceptible include pneumococci, types I, II, and III; beta-hemolytic streptococci, groups A and B; staphylococci; influenza bacilli; *E. coli;* and *Bacillus aerogenes.* The vapors have been shown to have viricidal effects against the viruses of influenza, meningopneumonitis, and psittacosis.

Toxicity studies have shown that because of the minute amounts (2 to 5 micrograms per liter of air) of triethylene glycol vapor required to produce maximal rates of bactericidal action the quantity retained by inhalation never approaches the toxic level.

Triethylene glycol is odorless and tasteless and is extremely hygroscopic. It has a low vapor pressure and thus vaporization is accomplished with a low amount of heat. The vapor has no deleterious effects on fabrics, books or other surfaces. The amount of vapor in the air should approach saturation. The closer saturation is approached the greater will be the extent of condensation of the vapor on particles suspended in the air and, apparently, the more rapid will be the destruction of air-borne organisms. A slight fog is an indication that the air is saturated. The amount of glycol vapor which saturates the air is determined by the relative humidity. It has been

found that the maximum rates of bactericidal action will be secured at humidities ranging from 20 to 50 per cent. The temperature also has an effect on the bactericidal action of the vapor. As the temperature was raised from 55 to 85° F. at a constant saturation level of glycol vapor there was found to be approximately a twofold increase in the rate of bactericidal action with each 15° F. rise in temperature. Another important factor to be considered is ventilation. Stagnation in air pockets should be avoided but under conditions of constant output of vapor the loss of vapor due to air exchange is exponential and, therefore, can represent an obstacle to the maintenance of adequate bactericidal concentration.

The authors also discussed features of design applicable to suitable vaporizing units. They also emphasized the importance of high standards of cleanliness and of personal hygiene as adjuncts to the use of triethylene glycol vapor for aerial disinfection.

The Effect of Terramycin in the Treatment of Amebiasis. Most, H. and Van Assendelft, F. *Ann. N. Y. Acad. Sci. 53:427* (1950). The antibiotic, terramycin, was given orally in divided doses in a total of 1 or 2 Gm. a day for a period of 10 days to 22 patients with colonic amebiasis resulting from infestation with *Endamoeba histolytica.* Among 12 of these patients stool speciments were examined daily and it was evident that degeneration of the parasites had begun within 48 hours. Within 3 days the parasites had usually disappeared. A relapse occurred in 1 patient after the discontinuation of treatment but the stools of the remaining 11 were negative through 1 to 93 days. The other 10 patients all had four negative stools at random during 14 to 17 days following treatment.

The authors also reported that terramycin eliminated *Endolimax nana* in 7 of 9 cases, and *Iodamoeba butschlii* in 2 cases. However, the antibiotic proved to be ineffective in 4 patients with hookworm infestations, 4 with infestations of *Trichuris trichuria,* 2 each with *Giardia lamblia, Schistomsoma mansoni, Hymenolepis nana,* and *Strongyloides stercoralis,* and in 1 patient with an infestation of *Trichomonas hominis.*

BOOK REVIEWS

Advanced Organic Chemistry. By George W. Wheland. Second edition 1949. John Wiley & Sons, Inc., New York.

The book contains 15 chapters of various length. Each chapter is subdivided and deals with the specific aspects of the general subject of the chapter. The book also contains 11 pages for author index. The footnoes are a scope of chemical literature up to about the 3rd month of 1949. These footnotes sometimes contain 2-4 references to the original literature thus serving as an excellent reference book.

The object of the book is to give an excellent theoretical treatise of chemistry. It is primarily concerned with modern structural theory and tries to support our present views. It also deals with the mechanism, the fundamental concepts and definitions of advanced chemical terms, elucidated by full structural formulae.

Especially good are the treatment of free radicals, strain theory and steric hindrance and structural theory.

The chapter on resonance slightly overstresses the possibilities of application in quantum mechanics in organic chemistry. The newer examples of steric effects are not included, neither is the steric inhibition of resonance pointed out with its pro and con. To a great extent, the following topics are missing: Ultraviolet and infrared spectroscopy, explicit discussion of the recent physical methods of structural investigation. Omitted are all Beilstein references. Omitted also is qualitative molecular treatment, and orientation in aromatic substitutions. Cycloalkane derivatives would be made clearer by using Kékule structures instead of single hexagons. However, these are all minor things and do not diminish the excellence of the book.

The book is much better written than similar books which treat the theoretical aspects of chemistry. Generally, the book is well written and gives an excellent picture to the student in his fourth college year. The explanations given are precise and generally exhaustive. It also contains very good criticism of the theoretical and some experimental material in the chapters. It emphasizes the experimental proof. From the point of view of theory only, it is the best recent book. It also explains certin misconceptions which date from previous times.

J. KORNER

Progress in Chromatography 1938-1947. By L. Zechmeister. 368 pages with 21 tables and 23 figures; John Wiley & Sons Inc., New York, New York, 1950.

In a previous monogram, Professor Zechmeister, in conjunction with Dr. L. Cholnoky, made available *Principles and Practice of Chromatography,* which discussed in a systematic manner the accomplishments in the embryonic field of chromatography up to 1938. Now, because of the frequent application of adsorption procedures to the study of problems in many fields and the introduction of new and very important methods of chromatography, Professor Zechmeister has reviewed the developments in this science from 1938 to 1947. He offers this review in his *Progress of Chromatography.*

Progress of Chromatography is divided into two sections, general and special. In the general section, the author begins with a general discussion of the principles and practice of chromatography. He then follows with a treatment of the separation of stereoisomers, the interaction between adsorbent and adsorbed substances, and exchange adsorption.

The special section specializes in the differences in the properties of various organic substances important in chromatography. Each one is covered in some detail, with a substantial bibliography listed for the worker. Among the organic substances subjected to specialized treatment are chlorophyll, porphyrins, bile pigments, carotenoids, synthetic dyestuffs, lipoids, carbohydrates, amino acids and peptides, sterols and steroids, heterocyclic compounds, alkaloids, vitamins, antibiotics and enzymes. Important applications of these substances are given. In the closing chapter, inorganic chromatography is surveyed, with emphasis on the separation of cations and anions and the fractionation of isotopes on ion exchangers.

Although Professor Zechmeister does not claim completeness in his survey of the bibliography because of the volume the literature of chromatography has reached, *Progress of Chromatography* serves as a welcome time-saver for the experimenter because of its valuable suggestions about methods, adsorbents or solvents which can be used within a certain class of compounds. The author presents critical analysis of all phases of his review, and presents an extensive bibliography for those interested in intensive research. Unfortunately, a new important phase of chromatography, the adsorption analysis of gases, has not been treated in this progress report.

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